



Bacterial Identification (Bac ID # 2)

Objective

To identify to the species level a single bacterial isolate by full-length rDNA gene sequencing (Bac ID #2)

Starting Materials

Bacterial isolation on agar plate

Methods

1. Picking of a single colony from the agar plate
2. Genomic DNA extraction with QIAamp DNA Mini Kit (Qiagen)
3. PCR of the full-length 16S rDNA gene using proprietary primers
4. Double strand sequencing of the full-length 16S rDNA gene using proprietary primers
5. Alignment of the consensus sequence against GenBank public database

Results

16S rDNA Consensus sequence (1474 bp):

```
CTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCG
GTAGAGAGAAGCTTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCC
TAGGAATCTGCCTGGTAGTGGGGGATAACGTTTCGAAACGGACGCTAATA
CCGCATACGTCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTAT
CAGATGAGCCTAGGTCCGATTAGCTAGTTGGTGGGGTAATGGCTCACCAA
GGCGACGATCCGTAACCTGGTCTGAGAGGATGATCAGTCACACTGGAAGT
AGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAA
TGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGG
ATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTTGCCTAATACGTAAC
TGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAG
CCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTAAGTGGGCGTAAA
GCGCGCGTAGGTGGTTTGTAAAGTTGGATGTGAAATCCCCGGGCTCAACC
TGGGAAGTGCATTCAAAGTACTGACTGACTAGAGTATGGTAGAGGGTGGTGG
AATTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAAGT
GCGAAGGCGACCACTGGACTAATACTGACACTGAGGTGCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACCGATGTCA
ACTAGCCGTTGGAAGCCTTGAGCTTTTGTAGTGGCGCAGCTAACGCATTAAG
TTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAGTCAAATGAATTGAC
GGGGGCCCCGCACAAGCGGTGGAGCATGTGGTTAATTGAAAGCAACGCGA
AGAACCTTACCAGGCCTTGACATCCAATGAACTTTCTAGAGATAGATTGG
TGCCTTCGGGAAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGT
TCGTGAGATGTTGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCTTAG
TTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACC
GGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGC
TACACACGTGCTACAATGGTTCGGTACAGAGGGTTGCCAAGCCGCGAGGTG
GAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCG
ACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTG
AATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGG
TTGCACCAGAAGTAGCTAGTCTAACCTTCGGGAGGACGGTTACCACGGTG
TGATTCATGACTGGGGTGAAGTCG
```



ACGT, INC.

800.557.ACGT (2248)
847.520.9162 phone
847.520.9163 fax
heron_yu@acgtinc.com

35 Waltz Drive
Wheeling, Illinois 60090
dnaseq@acgtinc.com
www.acgtinc.com

First 10 Blast Hits

Distribution of 100 Blast Hits on the Query Sequence

Sequences producing significant alignments:	Score (Bits)	E Value
gi 34494539 emb AJ581999.1 PLU581999 Pseudomonas lurida parti...	2922	0.0
gi 22658450 gb AY131215.1 Pseudomonas sp. Fa3 16S ribosomal RNA	2912	0.0
gi 21726937 emb AJ492829.1 PPO492829 Pseudomonas poae partial 16	2898	0.0
gi 21726939 emb AJ492831.1 PTR492831 Pseudomonas trivialis pa...	2890	0.0
gi 66878828 gb AY959122.1 Uncultured bacterium clone rRNA349...	2890	0.0
gi 20146542 gb AF374472.1 Pseudomonas costantinii 16S riboso...	2888	0.0
gi 54144894 gb AY756059.1 Pseudomonas sp. LBSA1 16S ribosoma...	2882	0.0
gi 32709414 gb AY259520.1 Pseudomonas sp. FB165 16S ribosomal R	2872	0.0
gi 47420202 gb AY599720.1 Pseudomonas sp. TB2-3-I 16S riboso...	2866	0.0
gi 7920705 gb AF255336.1 Pseudomonas tolaasii 16S ribosomal RNA	2864	0.0

Identification results

>gi|34494539|emb|AJ581999.1|PLU581999 Pseudomonas lurida partial 16S rRNA gene, type strain DSM 15835T

Length=1529
Score = 2922 bits (1474), Expect = 0.0
Identities = 1474/1474 (100%), Gaps = 0/1474 (0%)
Strand=Plus/Plus

```
Query 1 CTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGAGAGAA 60
|||||
Sbjct 12 CTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGAGAGAA 71

Query 61 GCTTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTG 120
|||||
Sbjct 72 GCTTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTG 131

Query 121 GGGGATAACGTTTCGAAACGGACGCTAATACCGCATAACGTCCTACGGGAGAAAGCAGGGG 180
|||||
Sbjct 132 GGGGATAACGTTTCGAAACGGACGCTAATACCGCATAACGTCCTACGGGAGAAAGCAGGGG 191

Query 181 ACCTTCGGGCCTTGCCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAT 240
|||||
Sbjct 192 ACCTTCGGGCCTTGCCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAT 251

Query 241 GGCTCACCAAGGCGACGATCCGTAACGGTCTGAGAGGATGATCAGTCACACTGGAAGT 300
|||||
Sbjct 252 GGCTCACCAAGGCGACGATCCGTAACGGTCTGAGAGGATGATCAGTCACACTGGAAGT 311

Query 301 AGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAG 360
|||||
Sbjct 312 AGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAG 371

Query 361 CCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTT 420
|||||
Sbjct 372 CCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTT 431

Query 421 GGGAGGAAGGGCAGTTGCCTAATACGTAACGTTTTCGAGTTACCGACAGAAATAAGCACC 480
|||||
Sbjct 432 GGGAGGAAGGGCAGTTGCCTAATACGTAACGTTTTCGAGTTACCGACAGAAATAAGCACC 491

Query 481 GGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTAC 540
|||||
```

Notice: This report is intended only for the addressee shown above and may contain confidential information. The data and other information in this report represent only the sample(s) analyzed and are rendered upon condition that is not to be reproduced without the approval from ACGT, Inc.

800.557.ACGT (2248)
847.520.9162 phone
847.520.9163 fax
heron_yu@acgtinc.com

35 Waltz Drive
Wheeling, Illinois 60090
dnaseq@acgtinc.com
www.acgtinc.com

```
Sbjct 492 GGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGAAGCGTTAATCGGAATTAC 551
Query 541 TGGGCGTAAAGCGCGCTAGGTGGTTTGTTAAGTTGGATGTGAAATCCCCGGGCTCAACC 600
          |||
Sbjct 552 TGGGCGTAAAGCGCGCTAGGTGGTTTGTTAAGTTGGATGTGAAATCCCCGGGCTCAACC 611
Query 601 TGGGAACTGCATTCAAACACTGACTGACTAGAGTATGGTAGAGGGTGGTGAATTTCTCTGT 660
          |||
Sbjct 612 TGGGAACTGCATTCAAACACTGACTGACTAGAGTATGGTAGAGGGTGGTGAATTTCTCTGT 671
Query 661 GTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGAC 720
          |||
Sbjct 672 GTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGAC 731
Query 721 TAATACTGACACTGAGGTGCGAAAGCGTGGGAGCAAACAGGATTAGATACCCTGGTAGT 780
          |||
Sbjct 732 TAATACTGACACTGAGGTGCGAAAGCGTGGGAGCAAACAGGATTAGATACCCTGGTAGT 791
Query 781 CCACGCCGTAAACGATGTCAACTAGCCGTTGGAAGCCTTGAGCTTTTAGTGGCGCAGCTA 840
          |||
Sbjct 792 CCACGCCGTAAACGATGTCAACTAGCCGTTGGAAGCCTTGAGCTTTTAGTGGCGCAGCTA 851
Query 841 ACGCATTAAAGTTGACCGCCTGGGAGTACGGCCGAAGGTTAAAACCTCAAATGAATTGAC 900
          |||
Sbjct 852 ACGCATTAAAGTTGACCGCCTGGGAGTACGGCCGAAGGTTAAAACCTCAAATGAATTGAC 911
Query 901 GGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTAC 960
          |||
Sbjct 912 GGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTAC 971
Query 961 CAGGCCTTGACATCCAATGAACTTTCTAGAGATAGATTGGTGCCTTCGGGAACATTGAGA 1020
          |||
Sbjct 972 CAGGCCTTGACATCCAATGAACTTTCTAGAGATAGATTGGTGCCTTCGGGAACATTGAGA 1031
Query 1021 CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGTAACG 1080
          |||
Sbjct 1032 CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGTAACG 1091
Query 1081 AGCGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCG 1140
          |||
Sbjct 1092 AGCGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCG 1151
Query 1141 GTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGC 1200
          |||
Sbjct 1152 GTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGC 1211
Query 1201 TACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCC 1260
          |||
Sbjct 1212 TACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCC 1271
Query 1261 CATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCG 1320
          |||
Sbjct 1272 CATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCG 1331
Query 1321 CTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTTACACACCGCC 1380
          |||
Sbjct 1332 CTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTTACACACCGCC 1391
Query 1381 CGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAACCTTCGGGAGGACGGT 1440
          |||
Sbjct 1392 CGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAACCTTCGGGAGGACGGT 1451
Query 1441 TACCACGGTGTGATTCATGACTGGGGTGAAGTCG 1474
          |||
Sbjct 1452 TACCACGGTGTGATTCATGACTGGGGTGAAGTCG 1485
```

Notice: This report is intended only for the addressee shown above and may contain confidential information. The data and other information in this report represent only the sample(s) analyzed and are rendered upon condition that is not to be reproduced without the approval from ACGT, Inc.

800.557.ACGT (2248) 35 Waltz Drive
847.520.9162 *phone* Wheeling, Illinois 60090
847.520.9163 *fax* dnaseq@acgtinc.com
heron_yu@acgtinc.com www.acgtinc.com

Conclusions

Sequence data obtained from analyzed sample have 100% identity with *Pseudomonas lurida*, GenBank Reference #AJ581999. Based on this complete identity, the sample is positively identified as *Pseudomonas lurida*.